EXPERIMENTAL RESTRAINT "ULCER" IN THE WHITE RAT I. Methods, Incidence of Lesions, and Modifications by Certain Technical and Pharmacodynamic Techniques

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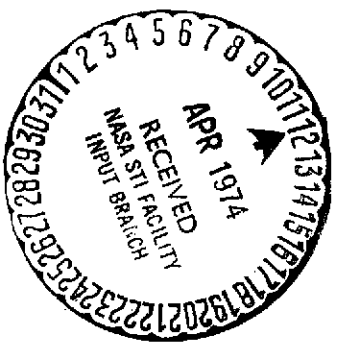

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16. Abstract

The article discusses the restraint ulcer in white rats and the incidence of lesions. Certain modifications of the method of study were made. This method does not involve surgery or pharmadynamic techniques, and, in addition, is relatively simple. Female white (Whistar) rats weighing 150-190 g were used.

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EXPERIMENTAL RESTRAINT "ULCER" IN THE WHITE RAT

I. Methods, Incidence of Lesions, and Modifications by Certain Technical and Pharmacodynamic Techniques

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It would appear that the study of experimental gastic ulcers /146* aims at producing lesions as close as possible in nature and development to the human ulcer.

Can we say that, of the hundreds of published techniques, such a result has frequently been obtained? Certainly not. As far as we can see, only the cinchophenic ulcer, just about, has achieved this end. But due to our present ignorance as to the method of action of atophan, this technique has posed more problems than it has helped to resolve.

In fact, investigations on the experimental gastric ulcer should permit Cruveilhier's ulcer not only to be reproduced, but to be better understood. For this reason, certain experimental methods are still of interest, even if they create only temporary or superficial lesions: any technique demonstrating a precise and irrefutable mechanism by which a gastric wall can be regularly injured, is worthy of attention. Here, we are probably creating ulcerations rather than ulcers: general syndromes where gastric alteration is only a subsidiary phenomenon rather than a true digestive disorder.

But our ignorance as to the detailed mechanism of the stomach ulcer in man is such that no line of research can be discarded a priori.

The technique we describe and discuss in the present article

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*Numbers in the margin indicate pagination in the foreign text.

escapes none of the above criticisms and considerations. Its main advantage is simplicity, the high number of positive results, and the fact that it does not involve surgery or pharmacodynamic action.

The discovery of the pathogenic effect of restraint on the white rat was a fortuitous one, when a method of studying gastric secretion by duodenal fistual was being developed [2]. After this we endeavored mainly to demonstrate the various pathogenic factors involved. This is why few pharmacodynamic studies on protection have been undertaken.

The restrain ulcer technique is related in some points to certain "stress" methods described by Selye [6]. But on other fundamental points, which we shall stress in particular, it differs, e.g., the immobility enforced on the animal's body. Moreover the interpretation that must be given to the restraint ulcer is clearly different from that of the stress ulcer: this is what the term "restraint" indicates, and why it was deliberately chosen as it indicates both the method of carrying out the experiment and one of the major pathogenic mechanisms - psychological restraint.

Techniques

We used female white (Whistar) rats weighing 150 to 190 g. They were removed at the last moment from their communal cages, where they live in groups of twenty. Since we do not breed the animals ourselves, we tried to experiment only with animals who had spent a minimum of twenty-four hours in our own laboratory.

The diet is immaterial, provided the rats are in satisfactory nutritional balance.

With no previous precautions, the animals are etherized for general anesthesia. During their sleep they are immobilized in a flexible grid with a maximum mesh of 1 cm, as follows: four holes are pierced at the location of the legs, which pass through effortlessly and protrude from the roots (the roots are not

restricted.) The legs are attached to one another in twos (front paws tied together; back paws tied together). Then the mesh is pressed down on each side such that the two ends overlap over the /147 animal's back, molding the circumference of the body without squeezing it. The device is tied to keep it in place. In the front, the mesh more or less supports the bearing of the head (Figure 1).

[Not reproducible]

Figure 1

The animal, immobilized in this fashion, is suspended horizontally by a burette holder such that its paws do not touch the ground. It remains in this position until sacrificed, i.e., for a period varying according to the experiment. There were two main durations: seven hours and twenty-four hours.

In all cases, from the beginning of restraint, 2.5 cm^3 physiological serum was injected into both the animal's rear legs.

One important detail: these restricted animals were in a laboratory where other experiments (on the rat in particular) were going on throughout the day.

Results

1. Frequency of Lesions after Seven and Twenty-Four Hours Restraint.

The detailed aspect of the lesions and assessment of their extent will be reported in a later section of this article: they

were multiple blackish ventricular ulcerations on the sides and greater curvature of the stomach (Figure 2).

[Not reproducible]

Figure 2.

The frequency distributions reported here do not take into account the greater or lesser number of lesions on one and the same stomach. Any animal in which a blackish ventricular ulceration could be observed with the naked eye was considered ulcerated. In practice the problem of borderline cases only came up very rarely.

a) Seven-hour restraint. Of 95 animals, 56 had gastric ulcerations, namely an incidence of 58.9%. The actual percentage (Snedcor) is between 49% and 69%.

b) Twenty-four hour restraint. 137 animals were in the experiment, of which 118 were affected with gastric ulceration. The incidence is 86.1% and the real percentage between 79% and 91%.

The difference between these two groups is statistically very significant: $p = 0.0000023$.

This shows that if a noteworthy incidence of lesions exists from the seventh hour, it increases with the duration of restraint.

2. Variations in Frequency of Lesions Obtained by Various Surgical and Pharmacodynamic Procedures.

Here we grouped the results of various tests [1,3,5] dictated either by the search for an analogy with the work of Selye, or for comparison with some of our preceding studies. In the present investigation one of the interests is to show the relative constancy of the phenomenon despite the addition of impact point factrs, both gastric and general.

These tests mainly used the seven-hour restraint to decrease the incidence of mortality during the experiment. Indeed, these treatments or operations weaken the animals considerably.

a) Adrenalectomy (seven-hour restraint). Bilateral adrenal- /148
ectomy is performed three to ten days before the restraint. Meanwhile survival is ensured by ad libitum administration, in the form of a drink, of solution containing 8 ppm sodium chloride and 150 ppm glucose.

Of the 49 rats in the experiment, 31 had gastric ulcerations, i.e., 67% the real percentage (Snedecor) being between 47 and 75. Thus there is no significant difference between this series and the control series.

b) Cortisone (seven-hour restraint). Cortisone was injected daily in a 10 mg dose in a uniform manner, whatever the weight of the rat. We recall that the weight of our animals is always between 150 and 190 g.

Two series were distinguished according to whether treatment lasted three or seven days.

1. Three-day series: of 46 rats, 19 had ulcerations, i.e., 41.3%. The real percentage (Snedcor) is between 28 and 57.

2. Seven-day series: of 81 rats, 28 had ulcers, or a percentage of 34.5% (real percentage bewteen 26 and 45).

Combining the two series, we see that 57 rats out of 127 had restraint ulcers, i.e., 37%; real percentage (Snedecor) between 29 and 46.

We can thus conclude that cortisone, under our conditions, reduces the frequency of restraint ulcers in a statistically significant manner. Prolongation of the treatment seems to be an efficiency factor.

c) Δ-1-cortisone (seven-hour restraint). The dose selected administered forcibly by mouth every day, was 2.5 mg per day. Here too, three- and seven-day series were run.

1. Three-day series: 11 rats out of 21 had restraint ulcerations.

2. Seven-day series: 17 rats out of 18 had lesions.

In total, 28 rats out of 39 were affected (71.7%) or a greater percentage than the control series.

d) Bilateral nephrectomy (seven-hour restraint). We felt it useful to perform nephrectomies for studying experimental ulcers (for reasons we shall not state here) in connection with studies on the ulcer by ligature of the pylorus (Shay's ulcer [4]). The vascular factors pay a preponderant role in this type of lesion, as they do in restraint ulcerations.

By reason of this similarity we also used this modification of technique in the present study.

A unilateral nephrectomy is performed between the twelfth and twenty-sixth day before restraint. The other kidney was removed /149 immediately after introduction into the mesh corsage.

Of 65 rats, 24 were affected with gastric ulceration (36.9%). The real percentage is between 24 and 48.

If we consider only the animals where nephrectomy preceded restraint by at least twenty days, the respective figures are 44 and 12 (27.2%).

Table 1

Technique	Number of animals	Number of ulcerated animals	Percent	Real percentage (Snedecor) between ... and ...
24-h restraint:				
Controls	137	118	86.1	79-91
Total vagotomies	39	20	51.3	32-66
> 10-day series	27	17	62.9	40-77
< 10-day series	12	3	25	5-55
7-h restraint:				
Controls	95	56	58.9	49-69
Adrenalectomy	63	43	68.2	54-80
10 mg cortisone:				
3 days	46	19	41.3	28-57
7 days	81	28	34.5	26-45
Total	127	47	37	29-46
Δ_1-cortisone:				
3 days	21	11	52.3	29-75
7 days	18	17	94.4	71-100
Total	39	28	71.7	54-85
Bilateral nephrectomy				
Total	65	24	36.9	24-48
> 20 days	44	12	27.2	15-43

In any event, the incidence of ventricular lesions out of all the nephrectomies is less, in a statistically significant way, than that of the control group ($p = 0.005$).

e) Vagotomy (twenty-four hour restraint). Vagotomy is performed bilaterally at the abdominal end of the esophagus. The trunks of the two nerves are easily distinguishable. In the rats we used there seemed to be no supradiaphragmetic spreading of the nerve, which would have rendered abdominal vagotomy illusory.

Restraint is established for twenty-four hours, six to twenty days after vagotomy. As we will state below, the mortality during the experiment is considerable (40%) and the animals which died spontaneously, usually before the seventeenth hour, had gastric ulcerations in the proportion of 50%.

Of the 39 rats which underwent the full twenty-four hour restraint, 20 had ventricular ulcerations (51.28%), or a real percentage of between 32 and 66). As the corresponding control

series gives a percentage of 86.1% (real percentage between 79 and 91) we can state that vagotomy significantly decreases the frequency of gastric lesions caused by restraint.

It appeared, however, that the intensity of this protection varied substantially according to the period between the date of vagotomy and that of restraint.

a) For ten days and more: we find 17 animals with ulcerations out of 27, i.e. 62.9% (real percentage between 40 and 77).

b) For nine days and less, we observe three animals out of 12 with ulcerations, i.e., 25%.

We do not know the reason for this difference. It seems that the vagotomized animals, as opposed to the other series, had wide variety in their reactions, both gastrically and in the general effects caused by restraint.

Discussion

The restraint ulcer is thus classified among the techniques causing acute multiple non-perforating ulceration of the glandular mucous membrane of the rat's stomach. In the absence of true ventricular ulcerations, we can observe purplish speckling, or punctiform bleeding areas in the same region. The keratinized portion, or rumen, is never affected.

The anatomic localization of the lesions thus places the restraint ulcer side by side with lesions caused by phenylbutazone, cortisone, and certain stress lesions (medullar section).

On the other hand, Shay's ulcer and protein shock ulcerations seem very different.

But the major distinguishing features of the technique reside in its simplicity and the apparent innocuity of the triggering factor, the early onset of the gastric lesions, and the increase of incidence with duration of restraint up to the twenty-four hour to a high and stable percentage of ulceration.

1. Simplicity and innocuity. The absence of operative shock or specific pharmacodynamic or general toxic action enables the involvement of the pathogenic stimulus to be quite clearly delimited. This can act either by itself (restraint) or by the reaction it causes in the animal which tries to free itself (release reaction), or by the two factors combined. We will approach the analysis of these phenomena in later articles.

2. Early onset. It should be emphasized that, from hour seven on, 58.9% $\pm$ 10 ulcerations exist. Staggered sacrifices showed that certain animals were able to create frank ulcerative lesions in three to four hours, but there is little point in working with such short times where the individual susceptibility of each rat plays a particularly important part causing wide heterogenity in the series.

3. High and very stable percentage of lesions over twenty-four hours. This feature is particularly valuable if we wish to use the restraint ulcer as a pharmacodynamic test, and all the more so as lethality is, as we will see, very low. The absence of fasting prior to restraint is probably one of the reasons for this.

The twenty-four hour period could, if working conditions demand, be cut down to twenty-three or even, if necessary twenty-two hours without the lesional incidence changing.

Thus, the difference in the percentage of ulceration between seven and twenty-four hours is considerable: 58.9% against 87.1% /150 ($p = 0.0000023$); indeed restraint periods must be strictly observed.

Conclusion

The interest of the technical variations touched on in the present article will mainly be substantiated in later articles. We will, however, bring out certain points:

a) The inaction of adrenalectomy shows simply that the stimulus does not pass via the axis between the hypophysis and the

adrenal gland. According to Selye, this is not a definitive argument against involvement of the "general adaptation syndrome".

b) The protective effect of cortisone, which becomes clearer and clearer as treatment progresses in length, is opposed to the inaction of Δ-1-cortisone. Thus cortisone is not acting as a flucocorticoid hormone. We will recall that, at any rate, neither substance has an excitosecretory effect on the stomach.

c) Vagotomy is the most classical method for reducing gastric secretion. It gives clear protection for the restraint ulcer, which raises the question of the role of the stomach's hydrochloric acid in the genesis of lesions.

d) Finally, nephrectomy was only used because it protects Shay's ulcer. It seems that in this case protection was exerted by the effect of surgery on gastric vascularization disturbances in this type of lesion.

The restraint ulcer protection afforded by nephrectomy is thus one approach to the problems of decreased resistance of the gastric wall.

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